

### REMARKS

Claims 1-41 were pending in this application. Claims 4-22 and 28-41 are canceled and claims 42-82 are added by this amendment. Upon entry of this amendment, claims 1-3, 23, and 42-82 will be pending in the application. Support for added claims 42-82 is found throughout the specification, including claims 1-41, as originally filed. Claims 42-82 correspond to original claims 1-41, but have been amended so as to obviate the objection under 37 CFR 1.75(c). There is no new matter in the added claims. A “marked-up version to show changes made” is attached to reflect the amendment of claims 1-3, and 23. A copy of all pending claims is attached to this reply.

### Restriction Requirement

Applicants respectfully request reconsideration of the pending restriction requirement, in view of new claims 42-82. Specifically, Applicants request that the new claims should be grouped as follow:

- III. Claims 42-46, are drawn to an oligonucleotide, a less than full length nucleotide sequence, classified in class 536, subclass 24.2 for example.
- IV. Claims 47-64, 69-82 are drawn to a recombinant nucleotide sequence comprising a concatenation of nucleotide coding for a plant protein reserve, expression vector, a method of transformation, and resulting transgenic plants, classified in class 800, subclass 278 for example.

V. Claims 65-68 are drawn to protein, classified in class 530, subclass 300 for example.

Applicants respectfully request that the restriction requirement be modified to reflect this submitted grouping paradigm. Upon such modification, Applicants elect the invention of Group III, claims 47-64 and 69-82, drawn to a recombinant nucleotide sequence comprising a concatenation of nucleotide coding for a plant protein reserve, expression vector, a method of transformation, and resulting transgenic plants.

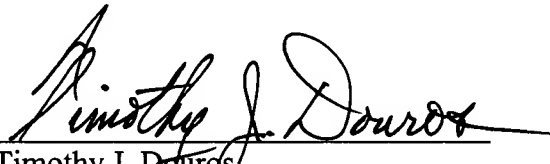
Pursuant to 37 C.F.R. 1.143, Applicants provisionally elect Group I, claims 1-3 and 23, as required in the January 31, 2001 action.

Enclosed is a petition to extend the period for replying for one month, to and including May 31, 2001. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date:

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"Version with markings to show changes made"

1. ~~An~~ oligonucleotide comprising at least one concatenation coding for a polypeptide

with formula  $(P-K)_n$ , where:

n is a whole number of 2 or more;

P represents a proline amino acid residue;

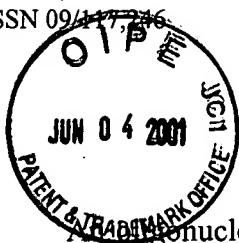
K represents a lysine amino acid residue;

the symbol "-" represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units also being bonded together by such bonds, for example peptide type bonds.

2. ~~An~~ The oligonucleotide according to claim 1, comprising a concatenation coding for a polypeptide with formula  $(P-K)_n$  where n is a whole number of 3 or more, and preferably n is equal to 4, 5, 6, 7, 8, 9, 10 or 15.

3. ~~An~~ The oligonucleotide according to claim 1 ~~or claim 2~~, comprising a concatenation coding for a polypeptide with formula  $(P-K)_n$ , in which the sequence of n (P-K) units is interrupted by one or more amino acid residues other than P or K residues.

23. A cloning and/or expression vector, ~~characterized in that it~~ which is one of plasmids pP20γZ (CNCM N° I-1640), pH30γZ or pH45γZ (CNCM N° I-1639).



### Pending Claims

1. An oligonucleotide comprising at least one concatenation coding for a polypeptide with formula  $(P-K)_n$ , where:
  - n is a whole number of 2 or more;
  - P represents a proline amino acid residue;
  - K represents a lysine amino acid residue;
  - the symbol "-" represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units also being bonded together by such bonds, for example peptide type bonds.
2. The oligonucleotide according to claim 1, comprising a concatenation coding for a polypeptide with formula  $(P-K)_n$  where n is a whole number of 3 or more, and preferably n is equal to 4, 5, 6, 7, 8, 9, 10 or 15.
3. The oligonucleotide according to claim 1, comprising a concatenation coding for a polypeptide with formula  $(P-K)_n$ , in which the sequence of n (P-K) units is interrupted by one or more amino acid residues other than P or K residues.
23. A cloning and/or expression vector, which is one of plasmids pP20 $\gamma$ Z (CNCM N° I-1640), pH30 $\gamma$ Z or pH45 $\gamma$ Z (CNCM N° I-1639).
42. An oligonucleotide comprising at least one concatenation coding for a polypeptide with formula  $(P-K)_n$ , where:
  - n is a whole number of 2 or more;
  - P represents a proline amino acid residue;
  - K represents a lysine amino acid residue;
  - the symbol "-" represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units also being bonded together by such bonds, for example peptide type bonds.

43. The oligonucleotide according to claim 42, comprising a concatenation coding for a polypeptide with formula  $(P-K)_n$  where  $n$  is a whole number of 3 or more, and preferably  $n$  is equal to 4, 5, 6, 7, 8, 9, 10 or 15.
44. The oligonucleotide according to claim 42, comprising a concatenation coding for a polypeptide with formula  $(P-K)_n$ , in which the sequence of  $n$  (P-K) units is interrupted by one or more amino acid residues other than P or K residues.
45. The oligonucleotide according to claim 42, wherein the concatenation coding for the polypeptide comprising the  $n$  (P-K) units is completed at its 5' end and/or at its 3' end by one or more codons coding, for example, for at least one lysine residue at the N-terminal extremity of the formed polypeptide.
46. The oligonucleotide according to claim 45, which codes for a polypeptide with formula (P-K), formula  $K-(P-K)_4$ , or with formula  $2K(P-K)_4$ .
47. A recombinant nucleotide sequence comprising a concatenation of nucleotides coding for a plant protein reserve, which further comprises an oligonucleotide according to any one of claims 42 to 46, inserted at one site of the nucleotide concatenation selected such that:
  - expression of the nucleotide sequence in a particular plant cell enables a modified protein reserve to be produced which is localized in that cell in a manner identical to or similar to the normal protein reserve which would be expressed in the same cell under the same conditions by the corresponding normal coding nucleotide concatenation; and/or
  - the modified protein reserve coded by the recombinant nucleotide sequence is immunologically recognized by antibodies produced against the corresponding normal protein reserve.
48. The nucleotide sequence according to claim 47, wherein the coding nucleotide concatenation codes for a protein reserve which is naturally low in lysine.

49. The nucleotide sequence according to claim 48, wherein the coding nucleotide concatenation codes for a protein reserve naturally produced by a plant for use in animal or human foodstuffs.
50. The nucleotide sequence according to claim 48, wherein the coding nucleotide concatenation codes for a protein reserve naturally produced by a plant from the cereal family.
51. The nucleotide sequence according to claim 48, wherein the coding nucleotide concatenation codes for a protein reserve naturally produced by a plant from the legume or crucifer family.
52. The nucleotide sequence according to claim 50, wherein the coding nucleotide concatenation codes for a maize protein reserve.
53. The nucleotide sequence according to claim 52, wherein the coding nucleotide concatenation codes for a protein reserve from the zein family.
54. The nucleotide sequence according to claim 53, wherein the coding nucleotide concatenation codes for a protein reserve which is maize  $\gamma$ -zein.
55. The nucleotide sequence according to claim 54, wherein the nucleotide concatenation coding for the maize  $\gamma$ -zein has the sequence shown in Figure 9.
56. The nucleotide sequence according to claim 48, wherein the coding nucleotide concatenation codes for a protein reserve of a plant selected from the following: soya, sunflower, tobacco, wheat, oats, alfalfa, rice, oilseed rape, sorghum, and *Arabidopsis thaliana*.

57. The nucleotide sequence according to claim 47, wherein the protein reserve encoded by the coding nucleotide concatenation is maize  $\gamma$ -zein, and wherein the oligonucleotide is inserted in place of or following a Pro-X domain or in a Pro-X domain naturally present in the maize  $\gamma$ -zein.
58. A recombinant nucleotide sequence, which comprises a nucleotide sequence according to claim 47 under the control of an expression promoter.
59. The recombinant nucleotide sequence according to claim 58, wherein the promoter is a specific promoter for a given cell tissue, for example a promoter which is specific for expression in grains, and/or in the leaves of plants.
60. The nucleotide sequence according to claim 58, wherein the expression promoter is that of maize  $\gamma$ -zein.
61. The nucleotide sequence according to claim 58, wherein the expression promoter is the promoter CaMV35S.
62. The nucleotide sequence according to claim 57, which codes for one of the polypeptides P20 $\gamma$ Z or H45 $\gamma$ Z with the sequences shown in Figures 11 and 10, respectively.
63. A cloning and/or expression vector, which comprises, at a site which is not essential for replication, a nucleotide sequence in accordance with claim 47.
64. A cloning and/or expression vector, which is one of plasmids pP20 $\gamma$ Z (CNCM N° I-1640), pH30 $\gamma$ Z or pH45 $\gamma$ Z (CNCM N° I-1639).
65. A polypeptide coded by a sequence according to claim 47.
66. A lysine-enriched modified maize  $\gamma$ -zein, which is coded by a nucleotide sequence according to claim 54.

67. A lysine-enriched modified maize  $\gamma$ -zein, the amino acid sequence of which is modified by at least one polypeptide with formula  $(P-K)_n$  or with formula  $2K(P-K)_n$ , where:
- n is a whole number of 2 or more;
  - P represents a proline amino acid residue;
  - K represents a lysine amino acid residue;
  - the symbol “-” represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units being bonded together by bonds, in particular peptide type bonds, said polypeptide having formula  $(P-K)_n$  or  $K-(P-K)_n$  being substituted for a sequence naturally present in the normal maize  $\gamma$ -zein or being inserted with deletion of one or more amino acids of the amino acid sequence for normal maize  $\gamma$ -zein, or being added to the normal  $\gamma$ -zein amino acid sequence, the insertion site for the polypeptide being selected such that:
    - when the modified lysine-rich  $\gamma$ -zein is produced in a host cell, in particular in a plant cell, it is localized in identical or similar manner to the normal maize  $\gamma$ -zein which would be produced under the same conditions in the same host cell; and/or
    - the modified maize  $\gamma$ -zein is recognized by antibodies directed against the normal maize  $\gamma$ -zein.
68. The modified maize  $\gamma$ -zein according to claim 67, which is the protein P20 $\gamma$ Z or the protein H30 $\gamma$ Z or the protein H45 $\gamma$ Z.
69. A recombinant host cell, which comprises a nucleotide sequence according to claim 47.
70. The host cell according to claim 69, which is a bacterium, for example *E. coli* or *Agrobacterium tumefaciens*.
71. The host cell according to claim 69, which is a plant cell.
72. The host cell according to claim 71, which is a plant seed cell.



73. The host cell according to claim 72, which is a cell from maize seed endosperm.
74. The host cell according to claim 73, which contains a nucleotide sequence according to claim 54, integrated in its genome in a stable manner.
75. The host cell according to claim 73, which produces a lysine-enriched modified maize  $\gamma$ -zein according to claim 67.
76. The host cell according to claim 71, which is a soya, sunflower, tobacco, wheat, oats, alfalfa, rice, oilseed rape, sorghum or *Arabidopsis cell*.
77. Seeds producing a polypeptide according to any one of claims 65 to 68.
78. A plant producing a polypeptide according to any one of claims 65 to 68.
79. The plant according to claim 78, which is a maize plant.
80. Seeds obtained from plants according to claim 78.
81. A method of producing plants or seeds expressing a modified protein reserve, which comprises the steps of:
  - a) transforming a plant cell with a nucleotide sequence according to claim 47, or a vector according to claim 63, under conditions enabling the modified protein reserve coded by the nucleotide sequence to be expressed in a stable and functional manner;
  - b) regenerating plants from the plant cell transformed in step a), to obtain plants expressing the modified protein reserve;
  - c) if necessary, obtaining seeds from the modified plants obtained in step b).
82. The method according to claim 81, wherein the plant is maize and the protein reserve is  $\gamma$ -zein.